III. Remarks and Conclusion

Applicants are filing this supplemental response to fully comply with the sequence listing requirements. Through diligence, it was noted that there was at least one sequence in Table 1 on page 15 of the specification. Accordingly, Applicants have updated the sequence listing and are submitting a paper copy and CD version through the US postal service. Further, Applicants are substituting the enclosed page 15 of the specification for the current page 15 of the specification. No new matter has been added.

Applicants respectfully request reconsideration of the rejections in light of this response and allowance of the case. The application is believed in a condition for allowance and Applicants respectfully request such action. Please call the below undersigned attorney for any assistance in securing allowance of this application. Please charge deposit account number 02-2334 for any required fees and to credit any credits.

Sincerely,

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EXAMPLES

Example 1.

Construction and analysis of recombinant VP5⁻ IBD virus

Construction of full length VP5⁻ clone of IBDV segment A.

To construct a VP5-negative IBDV, the *Eco*RI site immediately following the 3'-end of the full length cDNA of strain D78 segment A (pUC19FLAD78; Mundt and Vakharia, Proc. Natl. Acad. Sci. USA 93, 11131-11136, 1996) was deleted. An *Eco*RI - *Kpn*I fragment containing the T7 polymerase binding site followed by the complete segment A sequence was excised and inserted into *Eco*RI - *Kpn*I cleaved vector pUC18 after inactivation of the unique *Nde*I within the vector sequence resulting in plasmid pAD78/EK. Thereafter, the genomic region encompassing the initiation codon for VP5 was amplified in two pieces using primers A1F5' and VP5MutR, and VP5MutF and A2R, respectively (see Table 1 for sequence and location of primers). PCR fragments were cloned separately and were subsequently fused via a unique *Afl*II site which had been created by mutations within respective primers (see Fig. 2). An *Eco*RI - *Nde*I fragment containing the T7 polymerase binding site, and the 5'-part of segment A including the introduced mutations was excised and used to substitute the wild-type *Eco*RI - *Nde*I fragment in pAD78/EK to yield plasmid pAD78/VP5⁻. Of the three mutations introduced one altered the initiation methionine codon for VP5 into an arginine codon (Fig. 2).

Table 1: Sequence of oligonucleotide primers used for generating mutant constructs.

[a]Nucleotide sequence	Orientation	Designation	Nucleotide no.	SEQ ID NO:
AGAGAATTC*TAATACGACTCACTATA* GGATACGATCGGTCTGAC	+	A1F5`	1-18	9
TGGGCCTGTCACTGCTGTCACATGT	-	A2R	716 - 740	10
CATTGCTCTGCAGTGTGTAGTGAGC	-	A3R	338 - 362	11
CTACAACGCTATCCTTAAGGGTTA GTAGAG	+	VP5MutF	80 - 109	12
CTCTACTAACCCTTAAGGATAGCG TTGTAG	-	VP5MutR	80 - 109	13